REMARKS/ARGUMENTS

This response is being filed in conjunction with a Request for Continued Examination under the provisions of 37 CFR §1.114 and the appropriate fee. No new matter has been added. It is respectfully submitted that this response addresses all of the issues raised by the Examiner in the Office Action mailed August 25, 2005 and that the subject application is now in condition for allowance. Accordingly, reconsideration of the subject application is requested in view of the foregoing amendments and the following remarks.

Amendments to the Specification

Applicants have discovered errors in the amino acid sequences (SEQ ID NO. 2 and SEQ ID NO. 4) filed with the instant application in the USPTO both in the specification and in the sequence listing. The nucleic acid sequences as filed (SEQ ID NO. 1 and SEQ ID NO. 3) are correct, however the corresponding amino acid sequences have a typographical error wherein ASN residues were identified as ASP.

The typographical error occurred in amino acids 6, 13, 31, 39 and 43 of SEQ ID NO. 2 and amino acids 6 and 13 of SEQ ID NO. 4. As a point of clarification, the DNA codon for ASP is GAC/T and the DNA codon for ASN is AAC/T. Only one amino acid sequence can be generated from each DNA sequence and the correct DNA sequence was originally filed in this application. Therefore the correction of the amino acid sequences in this amendment does not constitute new matter. Applicants assert that the typographic error in the amino acid sequence was an inadvertent error.

A paper copy of a Substitute Sequence Listing is also submitted along with a Computer Readable Form (diskette) and a statement indicating that the Computer Readable form and the paper copy of the Substitute Sequence Listing represent the same listing.

Claim Rejections – 35 U.S.C. §103

Claims 26-59 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Chen et al. (Biotechnol. Prog. (1998), 14(5):667-71) in view of Brook et al. and Summers et al.

In order to establish a *prima facie* case of obviousness, three basic criteria must be met (MPEP §706.02(j)). First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference(s) or to combine reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art reference (or references) must teach or suggest all the claim limitations. Applicants submit that the Examiner has not established a *prima facie* case of obviousness for rejecting claims 26-59.

Chen et al. disclose the removal of mercury from contaminated water by using *E. coli* cells engineered to express mercury transport proteins on their surface and a GST-metallothionein fusion protein intracellularly. These engineered *E. coli* are immobilized in a cross-flow membrane bioreactor. Brook et al. disclose purification of a metallothionein-like metal binding protein from *Artemia*. Summers et al. disclose genetically engineered non-MT metal binding fusion proteins (chelons) and their production.

As presently claimed, the instant application provides devices and methods drawn to removing at least one metal from a substrate using a device comprised of a support having at least one substantially pure metallothionein (MT) protein bound to the support such that the substrate can be contacted with at least one MT protein and the metal binds to the at least one MT protein and is removed from the substrate.

As admitted by the Examiner, Chen et al. does not teach or disclose metallothionein from *Artemia*, nor does this reference teach the use of purified protein immobilized on a support and Brook et al. does not teach or disclose the use of the metallothionein in metal recovery devices or processes.

More specifically, Chen et al. discloses *E. coli* genetically engineered to express an Hg^{2+} transport system (the products of the *mer*T and *mer*P genes) on the bacterial cell surface and overexpress metallothionein (MT) as a glutathione S-transferase fusion protein (GST-MT) (page 667 of Chen et al.) within the bacteria cells. The genetically engineered cells, not any of the individual proteins, are immobilized in a bioreactor. The bioreactor thus described by Chen et al. transports, using the MerT and MerP proteins, Hg^{2+} from contaminated water, or another substrate source, into the genetically engineered *E. coli* where it is bound by the GST-MT. The bioreactor system of Chen et al. requires both the Hg^{2+} transport system, comprised of MerT and MerP proteins, and the GST-MT protein to sequester one heavy metal, Hg^{2+} in the *E. coli*. Chen et al. does not suggest or teach that MT proteins alone, much less MT proteins immobilized directly onto membranes, can be used to remove a variety of heavy metals from substrates. In fact the bioreactor system of Chen et al. uses the MerP and MerT proteins to remove the Hg^{2+} from the contaminated substrate and GST-MT to sequester the Hg^{2+}. Additionally, the bioreactor system of Chen et al. requires the expression of three proteins in the genetically engineered *E. coli* to remove mercury from contaminated substrates, rather than the single protein, a substantially pure MT protein, claimed in the instant application. The expression of solely MT protein in the system of Chen et al. renders the device inoperable as MT expressed within *E. coli* cells cannot remove mercury from a contaminated substrate as the MT does not contact the substrate.

The invention of the instant application claims the use of substantially purified MT alone for the removal of a variety of heavy metals from contaminated substrates. Chen et al. do not teach a single protein. Therefore the claims of the instant application have fewer limitations that the bioreactor system of Chen et al. and are thus not obvious over Chen et al. Based upon the teachings of Chen et al., a person of ordinary skill in the art would not be motivated to use fewer elements to achieve the invention of the instant application.

The Examiner has asserted that Brooks et al. teaches the purification of metallothionein from *Artemia* (Office Action mailed April 25, 2005 and restated in Final Office Action mailed August 25, 2005). The Applicants respectfully disagree.

Applicants' reading of Brooks et al. is that Brooks discloses the discovery and purification of a metallothionein-like zinc binding protein from *Artemia*. Brooks et al. additionally teaches a method of purifying a zinc-binding protein from *Artemia* which has characteristics of metallothionein and is identified as being metallothionein-like, however Brooks et al. does not disclose this protein as being metallothionein. Applicants therefore assert that Brooks et al. does not teach a substantially purified metallothionein protein from *Artemia*, as asserted by the Examiner in the Office Action mailed April 25, 2005. Therefore, a person of ordinary skill in the art would not be motivated to combine the teachings of Brooks et al. with the device of Chen et al. to obtain the invention of the instant application.

Summers et al. does not teach or suggest the use of a substantially pure metallothionein protein for removal of heavy metals from contaminated substrates. Summers et al. discloses an MT-free system. The chelon of the Summers et al. reference is comprised of "at least two metal binding domains from the MerR protein of the Tn21 mer (mercury resistance) operon" (column 5, lines 27-30). Summers et al. further states that the chelons described in his application are better than previously known metal binding proteins because those proteins have affinities and specificities for heavy metals which are lower although some forms of metallothionein bind divalent cadmium ions (Column 5, lines 40-46). Summers et al. disclosed that the binding affinity of chelons for mercury extents from as low as 10^{-9} M and up to about 1M. (Column 8, lines 15-18) Therefore, by stating that MTs are inferior to chelons with regard to metal binding affinity, the disclosure of Summers et al. would discourage persons of ordinary skill in the art from the use of MTs in metal binding devices. Therefore, a person of ordinary skill in the art would not be motivated to combine the teachings of Summers et al. and Brooks et al. with the device of Chen et al. to obtain the invention of the instant application.

Therefore, Applicants respectfully assert that the Examiner has not established a *prima facie* case of obviousness with regard to claims 26-59. The combination of Chen et al. with Brooks et al. and Summers et al. do not render the claims of the instant application obvious. First, there is no suggestion or motivation to combine the cited

references. The fact that zinc binding metallothionein-like proteins were known (Brooks et al.) does not suggest that a substantially pure MT protein could be substituted for the genetically engineered bacteria producing three different mercury transport and binding proteins of Chen et al., even with the knowledge that Summers et al. disclose immobilizing genetically engineered chelons on solid supports. Second, there is no expectation of success for using a substantially pure MT protein immobilized on a solid support to remove a plurality of different metals from substrates when the cited prior art discloses using (1) a combination of three different mercury transport and binding proteins genetically engineered into *E. coli* immobilized in a bioreactor (Chen et al.) to remove just mercury from substrates, or (2) artificial fusion proteins (chelons) generated from mercury transport proteins which are immobilized on solid supports. Third, the combination of the three cited references do not teach all the claim limitations of the instant application. The combination of Chen et al., Brooks et al. and Summers et al do not teach or suggest the use of a substantially purified MT protein which is immobilized for the removal of a variety of heavy metals from substrates and therefore do not render the claims of the instant application obvious over the cited prior art.

Applicants respectfully request that the Examiner withdraw the rejection of claims 26-59 under 35 U.S.C. §103(a).

Conclusion

For the foregoing reasons, Applicant believes pending claims 26-59 are in condition for allowance and respectfully requests that a timely Notice of Allowance be issued in this case.

The Commissioner is authorized to charge any fee which may be required in connection with this Amendment to deposit account No. 50-3207.

Respectfully submitted,

Dated: 11/8/05

Michelle S. Glasky, Ph.D.
Registration No. 39,645

PRESTON GATES & ELLIS, LLP
1900 Main Street, Suite 600
Irvine, California 92614-7319
Telephone: (949) 253-0900
Facsimile: (949) 253-0902

CUSTOMER NUMBER: 45,200